

CLAIMS

What is claimed is:

- 5 1. A method of determining the genotype of a sample polynucleotide having at least a first variant site, comprising:
 - amplifying at least a portion of the sample polynucleotide to obtain first amplicons, the first amplicons including the first variant site;
 - combining the first amplicons with first and second different
 10 polynucleotide controls, the first and second polynucleotide controls differing by at least one base therealong, the position of the at least one differing base corresponding to the first variant site of the sample polynucleotide;
 - preparing a plurality of first duplexes, each of at least some of the first duplexes comprising (i) a polynucleotide strand of one of the first
 15 amplicons and (ii) a complementary polynucleotide strand of the first polynucleotide control;
 - preparing a plurality of second duplexes, each of at least some of the second duplexes comprising (i) a polynucleotide strand of one of the first amplicons and (ii) a complementary polynucleotide strand of the second
 20 polynucleotide control;
 - subjecting the first and second duplexes to temperature gradient electrophoresis (TGE) to obtain first and second electrophoresis data; and
 - determining the genotype of the first variant site of the sample polynucleotide based on the first and second electrophoresis data.
- 25 2. The method of claim 1, wherein determining the genotype of the sample polynucleotide comprises determining a number of peaks present in the first electrophoresis data and a number of peaks present in the second electrophoresis data.
- 30 3. The method of claim 1, wherein the first duplexes and second duplexes are subjected to TGE along first and second different separation lanes.
4. The method of claim 1, wherein the first and second polynucleotide controls are wild-type polynucleotides.

5. The method of claim 1, comprising:

amplifying at least a second different portion of the sample polynucleotide to obtain second amplicons, the second amplicons including a second variant site of the sample polynucleotide;

5 combining the second amplicons with third and fourth different polynucleotide controls, the third and fourth polynucleotide controls differing by at least one base therealong, the position of the at least one differing base corresponding to the second variant site of the sample polynucleotide;

10 preparing a plurality of third duplexes, each of at least some of the third duplexes comprising (i) a polynucleotide strand of one of the second amplicons and (ii) a complementary polynucleotide strand of the third polynucleotide control;

15 preparing a plurality of fourth duplexes, each of at least some of the fourth duplexes comprising (i) a polynucleotide strand of one of the second amplicons and (ii) a complementary polynucleotide strand of the fourth polynucleotide control;

subjecting the third and fourth duplexes to temperature gradient electrophoresis (TGE) to obtain third and fourth electrophoresis data; and

20 determining the genotype of the second variant site of the sample polynucleotide based on the third and fourth electrophoresis data.

6. The method of claim 5, wherein at least one of the first and second duplexes has a size that differs from at least one of the third and fourth duplexes and wherein subjecting the first and second duplexes to TGE and subjecting the third and fourth duplexes to TGE comprise simultaneously subjecting at least
25 3 duplexes of the first, second, third, and fourth duplexes to TGE along the same separation lane.

7. The method of claim 6, wherein at least one of the first and second duplexes has a size that differs from at least one of the third and fourth duplexes by at least 20 base pairs.

30 8. The method of claim 1, comprising:

amplifying at least a first portion of a second different sample polynucleotide to obtain second amplicons, the second sample polynucleotide

comprising a second variant site, the second amplicons including the second variant site of the sample polynucleotide;

combining the second amplicons with third and fourth different polynucleotide controls, the third and fourth polynucleotide controls differing by at least one base therealong, the position of the at least one differing base corresponding to the second variant site of the second sample polynucleotide;

preparing a plurality of third duplexes, each of at least some of the third duplexes comprising (i) a polynucleotide strand of one of the second amplicons and (ii) a complementary polynucleotide strand of the third polynucleotide control;

preparing a plurality of fourth duplexes, each of at least some of the fourth duplexes comprising (i) a polynucleotide strand of one of the second amplicons and (ii) a complementary polynucleotide strand of the fourth polynucleotide control

subjecting the third and fourth duplexes to temperature gradient electrophoresis (TGE) to obtain third and fourth electrophoresis data; and

determining the genotype of the second variant site of the sample polynucleotide based on the third and fourth electrophoresis data.

9. The method of claim 8, wherein at least one of the first and second duplexes has a size that differs from at least one of the third and fourth duplexes and wherein subjecting the first and second duplexes to TGE and subjecting the third and fourth duplexes to TGE comprise simultaneously subjecting at least 3 duplexes of the first, second, third, and fourth duplexes to TGE along the same separation lane.

10. The method of claim 9, wherein at least one of the first and second duplexes has a size that differs from at least one of the third and fourth duplexes by at least 20 base pairs.

11. A method for determining the genotype of a sample polynucleotide, comprising:

providing first and second polynucleotide controls, the first and second polynucleotide controls differing by at least one base therealong, the position of the differing base corresponding to a position of a variant site of

the sample polynucleotide;

combining a first amount of the sample polynucleotide with the first polynucleotide control to prepare a first mixture, each of the sample polynucleotide and the first polynucleotide control comprising a polynucleotide strand sufficiently complementary to form a duplex with a polynucleotide strand of the other of the sample polynucleotide and first polynucleotide control;

forming first duplexes, at least some of the first duplexes comprising a strand of the sample polynucleotide and a strand of the first polynucleotide control;

combining a first amount of the sample polynucleotide with the second polynucleotide control to prepare a second mixture, each of the sample polynucleotide and the second polynucleotide control comprising a polynucleotide strand sufficiently complementary to form a duplex with a polynucleotide strand of the other of the sample polynucleotide and second polynucleotide control;

subjecting the first and second mixtures to temperature gradient electrophoresis to obtain first and second electrophoresis data; and

determining the genotype of the sample polynucleotide based on the first and second electrophoresis data.

12. The method of claim 11, wherein determining the genotype of the sample polynucleotide comprises determining a number of peaks present in the first electrophoresis data and a number of peaks present in the second electrophoresis data.

13. The method of claim 11, wherein both the first and second polynucleotide controls are homozygous.

14. The method of claim 11, wherein the sample polynucleotide comprises an amplicon prepared by amplifying a first double stranded polynucleotide.

15. A method for determining the genotype of a first variant site of a first sample polynucleotide, comprising:

providing amplicons of the of the first sample polynucleotide,

the amplicons including the first variant site;

subjecting a first portion of the amplicons to denaturing and annealing to prepare a first mixture;

5 providing a first polynucleotide control, the first polynucleotide control comprising at least one polynucleotide strand able to form a duplex with a polynucleotide strand of at least one of the amplicons, the first polynucleotide control having a base corresponding to the first variant site of the sample polynucleotide, the identity of the base being known;

10 combining a second portion of the amplicons with the first polynucleotide control to prepare a second mixture

subjecting the second mixture to denaturing and annealing to prepare a third mixture;

subjecting the first mixture to temperature gradient electrophoresis (TGE) to obtain first electrophoresis data;

15 subjecting the second mixture to temperature gradient electrophoresis (TGE) to obtain second electrophoresis data; and

wherein the first and second electrophoresis data are indicative of the genotype of the first variant site of the first sample polynucleotide.

16. The method of claim 15, comprising determining the genotype of the first
20 variant site of the sample polynucleotide based on the first and second electrophoresis data.

17. The method of claim 15, wherein the step of subjecting a first portion of the amplicons to denaturing and annealing to prepare a first mixture is performed prior to introducing the amplicons to an electrophoresis separation lane.

25 18. The method of claim 15, wherein the step of subjecting the second mixture to denaturing and annealing to prepare a third mixture is performed prior to introducing the second mixture to an electrophoresis separation lane.

19. The method of claim 15, wherein the sample polynucleotide comprises a second variant site and the method comprises:

30 providing second amplicons of the of the first sample polynucleotide, the second amplicons including the second variant site;

subjecting a first portion of the second amplicons to denaturing and annealing to prepare a fourth mixture;

5 providing a second polynucleotide control, the second polynucleotide control comprising at least one polynucleotide strand able to form a duplex with a polynucleotide strand of at least one of the second amplicons, the second polynucleotide control having a base corresponding to the second variant site of the first sample polynucleotide, the identity of the base being known;

10 combining a second portion of the second amplicons with the second polynucleotide control to prepare a fifth mixture

subjecting the fifth mixture to denaturing and annealing to prepare a sixth mixture;

subjecting the fourth mixture to temperature gradient electrophoresis (TGE) to obtain third electrophoresis data;

15 subjecting the sixth mixture to temperature gradient electrophoresis (TGE) to obtain fourth electrophoresis data; and

wherein the third and fourth electrophoresis data are indicative of the genotype of the second variant site of the first sample polynucleotide.

20. The method of claim 19, comprising determining the genotype of the first variant site of the sample polynucleotide based on the first and second electrophoresis data.

21. The method of claim 19, wherein the step of subjecting a first portion of the amplicons to denaturing and annealing to prepare a first mixture is performed prior to introducing the amplicons to an electrophoresis separation lane.

25 22. The method of claim 19, wherein the step of subjecting the second mixture to denaturing and annealing to prepare a third mixture is performed prior to introducing the second mixture to an electrophoresis separation lane.

23. The method of claim 15, comprising:

30 providing second amplicons of a second sample polynucleotide, the second amplicons including a second variant site of the second sample polynucleotide;

subjecting a first portion of the second amplicons to denaturing and annealing to prepare a fourth mixture;

providing a second polynucleotide control, the second polynucleotide control comprising at least one polynucleotide strand able to form a duplex with a polynucleotide strand of at least one of the second amplicons, the second polynucleotide control having a base corresponding to the second variant site of the second sample polynucleotide, the identity of the base being known;

combining a second portion of the second amplicons with the second polynucleotide control to prepare a fifth mixture

subjecting the fifth mixture to denaturing and annealing to prepare a sixth mixture;

subjecting the fourth mixture to temperature gradient electrophoresis (TGE) to obtain third electrophoresis data;

subjecting the sixth mixture to temperature gradient electrophoresis (TGE) to obtain fourth electrophoresis data; and

wherein the third and fourth electrophoresis data are indicative of the genotype of the second variant site of the second sample polynucleotide.

24. The method of claim 23, comprising determining the genotype of the first variant site of the sample polynucleotide based on the first and second electrophoresis data.

25. The method of claim 23, wherein the step of subjecting a first portion of the amplicons to denaturing and annealing to prepare a first mixture is performed prior to introducing the amplicons to an electrophoresis separation lane.

26. The method of claim 23, wherein the step of subjecting the second mixture to denaturing and annealing to prepare a third mixture is performed prior to introducing the second mixture to an electrophoresis separation lane.